

Regulation of Stem Cell Pluripotency and Neural Differentiation by Lysophospholipids

Stuart M. Pitson^{a,b} Alice Pébay^c

^aDivision of Human Immunology, Centre for Cancer Biology, SA Pathology, and

^bSchool of Molecular and Biomedical Science, University of Adelaide, Adelaide, S.A., and

^cCentre for Neuroscience and Department of Pharmacology, The University of Melbourne, Parkville, Vic., Australia

Key Words

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Abstract

Lysophospholipids are bioactive signalling molecules able to act through the binding of their specific G-protein-coupled receptors to exert pleiotropic effects on a wide range of cells. The most widely studied signalling lysophospholipids are lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). LPA and S1P have been identified to have widespread developmental, physiological and pathological actions in the central nervous system and more recently have been shown to induce biological effects on various stem cell types. This review aims to summarise the current knowledge on LPA and S1P regulation of embryonic and neural stem cell biology.

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Introduction

The derivation of human embryonic stem cells (hESCs) in 1998 opened up many exciting new opportunities to further investigate the therapeutic potential of stem cells [1]. Mouse and human ESCs share many properties: they originate from a pluripotent population of cells within the pre-implantation embryo, are karyotypically normal, can be propagated indefinitely (self-renewal) and can differentiate in vitro and in vivo into cells representative of the three embryonic germ layers [for review, see 2]. Although very similar, mouse and human ESCs also show major differences. In particular, the underlying mechanisms of mouse and human ESC maintenance of pluripotency appear to be different [for review, see 3, 4].

Neural stem cells give rise to neural and neuronal progenitor cells. While neural stem/progenitor cells (NS/PCs) can differentiate into neurons, astrocytes and oligodendrocytes, neuronal progenitor cells, or neuroblasts, differentiate only into neurons. Oligodendrocyte progenitor cells (OPCs) derived from their neural progenitors share properties with both stem cells and progenitor cells and give rise to oligodendrocytes, which allow myelination within the central nervous system (CNS) [for review, see 5]. During development, NS/PCs contribute

to the neurogenesis of the CNS. In the adult, NS/PCs are located predominantly in neurogenic regions of the CNS, such as the subventricular zone and hippocampus but are also scattered throughout the CNS. NS/PCs can migrate to areas of injury, and also to tumours, and differentiate into neuronal and glial cell types [6], thus are likely to contribute to the repair of damaged tissue. NS/PCs have been extensively studied with the aim of using endogenous and/or donor NS/PCs to replace neurons and restore circuitry in a neurodegenerative microenvironment.

The factors that control the regulation of stem cell survival, proliferation, migration and differentiation are still emerging. Considerable evidence now exists demonstrating the potent effects of lysophospholipids, and in particular lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), on the biology of stem cells. This review aims to summarise the current knowledge of the regulation of embryonic and neural stem cell maintenance and differentiation by LPA and S1P.

Lysophospholipid Metabolism and Signalling

Lysophospholipids are simple phospholipids with only a single O-acyl chain. Despite their generally simple structure, lysophospholipids appear to be involved in regulating a wide array of cellular processes through their functions as extracellular ligands for cell surface receptors and as intracellular second messengers. Biological effects have been described for a wide variety of phospholipids and lysophospholipids, including sphingomyelinase, platelet-activating factor, alkyl glycerol phosphate and cyclic phosphatidic acid. The most extensively studied bioactive lysophospholipids, however, are LPA and S1P.

LPA and S1P Metabolism

Various pathways exist to facilitate the formation of LPA. Most of the LPA in serum and plasma appears to arise from the activity of autotaxin, a secreted lysophospholipase D that generates LPA from lysophospholipids such as lysophosphatidylcholine, lysophosphatidylserine or lysophosphatidylethanolamine released from activated platelets [7]. LPA can also be generated extracellularly from the deacylation of phosphatidic acid by the activity of secreted phospholipases A₁ and A₂ [for review, see 8]. A similar route for LPA generation catalysed by intracellular phospholipases A₁ and A₂ also occurs within cells which appears responsible for the high levels of LPA

stored in platelets [8]. Although less abundant, LPA can also arise from phosphorylation of monoacylglycerol by monoacylglycerol kinase [9] or acylation of glycerol 3-phosphate by glycerol 3-phosphate acyltransferase [10].

In contrast to that of LPA, generation of S1P occurs by only a single route via the phosphorylation of sphingosine by the sphingosine kinases (SphKs), of which 2 have been identified in mammals (SphK1 and SphK2). Growth factor or cytokine-induced activation of SphK1 results in re-localisation of this enzyme to the plasma membrane where it appears to be the major source of S1P under these conditions [11, 12]. Notably, however, knockout mouse studies indicate that both SphK1 and SphK2 may play an equivalent role in maintaining plasma S1P levels [13, 14]. The cellular source of circulating S1P has been the subject of considerable recent debate with studies suggesting platelets [15], erythrocytes [16] and vascular endothelial cells [17] as major sources of S1P. Release of this intracellular S1P has been thought to be mediated by ATP-binding cassette (ABC) transporters, specifically ABCC1 in mast cells [18], although recent zebrafish genetic studies have identified a novel sphingolipid transporter, spinster 2 (also called two of hearts) that appears to also function in this manner [19, 20]. Interestingly, SphK1 can also be secreted where it may generate S1P extracellularly, although this appears likely to generate only a minor proportion to the pool extracellular S1P [21–23].

Cellular Signalling by LPA and S1P

Plasma levels of LPA and S1P dramatically increase after injury due to their (or their precursor's) release from activated platelets, leading to a variety of responses in tissues, including in the CNS. These lysophospholipids have effects on most cells, eliciting responses to alter proliferation, survival, migration and differentiation. Many of these effects are mediated through widely expressed G-protein-coupled receptors (GPCRs) for these lysophospholipids [24]. Five S1P receptors (S1P_{1–5}) have been established, while up to 7 receptors for LPA have been identified, including LPA_{1–5}, and the more recently discovered GPCRs P2Y5 [25–27] and GPR87 [28]. A further GPCR, P2Y10, has also been recently proposed to have both S1P and LPA as ligands [29], although further characterisation of this receptor is required.

The S1P and LPA receptors are differentially expressed and linked to different G proteins, allowing these lysophospholipids to elicit a variety of cell-specific responses through the activation of classic G_i, G_q, G₁₂, and possibly G_s, signalling pathways [24, 30, 31]. Depending on the receptors present, many of the signalling effects of LPA

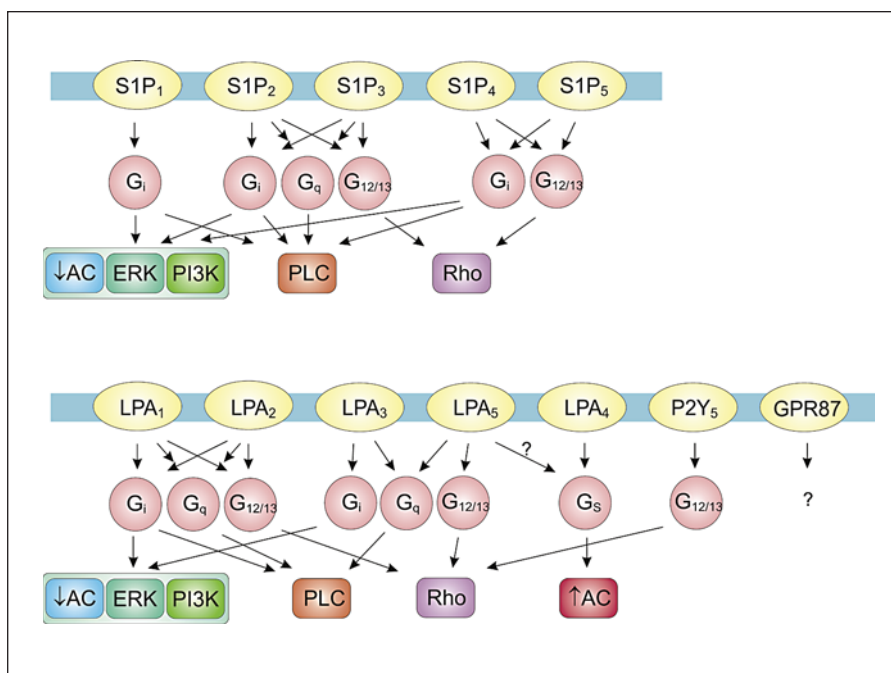


Fig. 1. General signalling pathways regulated by S1P and LPA. Association of S1P and LPA receptors with G-protein families and the general signalling pathways regulated are indicated.

and S1P are mediated through the activation of extracellular signal-regulated kinases 1/2 (ERK1/2), phospholipase C (PLC), and small GTPases, as well as through calcium mobilisation, and the activation or inhibition of adenylyl cyclase (AC; fig. 1). While many of the downstream pathways leading from each individual lysophospholipid receptor have been elucidated, it is becoming increasingly clear that lysophospholipid-regulated biological outcomes are commonly dependent on integration of signals from multiple lysophospholipid receptors as well as their well-established cross-talk with cytokine and growth factor receptors, such as the platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) receptors [22, 32–35]. Thus, the challenge remains to understand how the integration of these divergent signalling pathways leads to the fine control of cellular processes.

In addition to GPCR-mediated signalling, LPA can also act as an intracellular second messenger via its association with the nuclear transcription factor, peroxisome proliferator-activated receptor γ (PPAR γ) [36]. PPAR γ regulates genes involved in vascular inflammation, adipocyte differentiation, as well as glucose and fatty acid metabolism [37], and binding of LPA appears to stimulate its transcriptional activity. An intracellular second messenger role for S1P has long been proposed, but to date no direct intracellular targets of S1P have been identified and remains a future challenge.

LPA/S1P Signalling in Embryonic Stem Cells

LPA/S1P Signalling in Mouse Embryonic Stem Cells

Mouse ESCs (mESCs) express LPA and S1P receptors with variation between mESC lines which might reflect differences in the strains used, or in the experimental conditions used to derive or maintain mESCs [38–40]. All 5 S1P receptors are expressed in R1 mESCs [39], while S1P₄ is not found to be expressed in CGR8 and ES-D3 mESCs [40]. LPA_{1–3,5} have been described in R1 mESCs [39, 41]. Both LPA and S1P are positive regulators of mESC proliferation with data showing that LPA increases the mESC proliferation rate [41] and that antagonists of S1P_{1–2} inhibit basal mESC proliferation [40]. In mESCs, the main pathway allowing maintenance of pluripotency appears to be through the activation of the JAK/STAT3 pathway, leading to the transcription of self-renewal genes [for review, see 42]. Although demonstrated in other cell types [43, 44], stimulation of STAT3 by either LPA or S1P in mESCs has not yet been described. However, Todorova et al. [41] recently demonstrated that LPA induces mESC proliferation through the PLC/calcium mobilisation-induced expression of *c-myc*. As both LPA and S1P stimulate ERK1/2 phosphorylation [40, 45], a pathway involved in mESC proliferation but also differentiation [42, 46], these bioactive lipids can also potentially be regulators of mESC differentiation. Indeed, in parallel to

its effect on proliferation, S1P promotes mESC-embryoid body (EB) differentiation towards cardiomyocytes [47]. However, ERK1/2 activation by LPA induces the expression of the early gene *c-fos* which would suggest a role in maintenance rather than in differentiation [45]. JNK1/2 phosphorylation is also stimulated by LPA, yet its biological significance remains to be characterised [45]. Since pharmacological tools for blocking/stimulating specific aspects of lysophospholipid signalling are only now becoming commercially available, little is currently known of LPA/S1P receptor subtype-specific effects in mESCs. Yet activation of the ERK1/2 pathway by S1P seems to be mediated by S1P₅/G_i and not involved in the regulation of basal mESC proliferation [40].

Endogenous sphingolipid metabolism is likely to play a role in ESC maintenance and differentiation. Ceramide is a precursor of sphingosine and its levels are closely related to those of S1P. Ceramide induces the binding of prostate apoptosis response protein 4 (PAR-4) to the atypical protein kinase C ζ [48], an effect responsible for ceramide-induced apoptosis in cells showing elevated levels of PAR-4. As S1P antagonises ceramide-induced apoptosis [49], it is likely to protect any cell type with high levels of PAR-4 from ceramide-induced apoptosis [50]. In mESCs, the endogenous levels of ceramide are low, increase upon EB formation until the early phase of NS/PC differentiation and finally decrease during the following steps of neural differentiation [48]. Furthermore, there is an asymmetric distribution of PAR-4 and endogenous ceramide levels in cells within EBs, with PAR-4 being more expressed within the ESC of the EBs [48]. This restrictive partitioning suggests a specificity of apoptosis by ceramide on ESCs while the NS/PCs further proliferate or differentiate [48], which, as a consequence, reduces the amount of ESCs and enriches EBs in NS/PCs [51]. EBs and neurospheres are often considered a better source of cells than undifferentiated ESCs for stem cell engraftment, as they have less potential to induce the formation of teratomas, due to their reduced differentiation potential. However, the inoculation of EBs or neurospheres in vivo can always be contaminated by less committed cells. Thus, a selective-induced apoptosis of the less committed cells within EBs by ceramide has the potential of preventing teratoma formation and enriching EBs in NS/PCs when injected in vivo [51].

LPA/S1P Signalling in Human Embryonic Stem Cells

Some of the most potent protocols for long-term hESC culture require the presence of a feeder cell layer of mouse embryonic fibroblasts (MEFs) in serum or Knockout

Serum Replacement[®] media (hESC media). hESCs and MEFs have different profiles of lipid content and basal sphingolipid metabolism, with sphingosine, S1P, sphingomyelin and glucosylceramide found at lower levels in hESCs than in MEFs [52], suggesting that MEFs may release bioactive sphingolipids in the culture medium. This could account for some of the MEF-mediated effects on hESCs. hESCs are target cells of LPA and S1P, as their receptors LPA₁₋₅ and S1P₁₋₃ have been found to be expressed on various hESC lines [53–55]. No biological effect of LPA on hESCs has been described so far [53], and contrary to what was described in mESCs, we have not observed modification of the intracellular calcium concentration in hESCs treated with LPA [Wong and Pebay, unpublished data]. While S1P does not substantially prevent spontaneous differentiation of hESCs, its co-incubation with PDGF allows the long-term maintenance of hESCs in vitro in the absence of serum and in the presence of MEFs [53]. This long-term maintenance of hESCs by S1P and PDGF is at least G_i-, ERK1/2- and SphK-dependent [53]. S1P and PDGF appear to act on the different levels of hESC maintenance: pluripotency, proliferation and survival, through the activation of specific signalling pathways [53, 56]. Exposure to S1P results in sustained activation of the ERK1/2 pathway (at least 5 h) but not the PI3K/Akt pathway, while PDGF stimulates both ERK1/2 and PI3K/Akt pathways [56, 57]. Interestingly, although the Smad2/3 pathway appears to be essential to hESC maintenance in different culture systems [58], neither S1P nor PDGF modifies Smad2 phosphorylation even after 2 h of incubation [56]. We have also found that S1P and PDGF stimulate the phosphorylation of p38 and, less potently, JNK1/2, although the physiological consequences of this have not yet been established (fig. 2). S1P stimulates proliferation and inhibits apoptosis and necrosis of hESC when added to a MEF-conditioned medium supplemented in basic fibroblast growth factor (bFGF) [54]. As these data were obtained in the presence of bFGF, whether this effect is indeed independent of bFGF remains to be assessed. The sole application of S1P is anti-apoptotic to hESCs, an effect abolished by inhibition of the ERK1/2 or PI3K/Akt pathways, but not altered by the inhibition of the mammalian target of rapamycin [56]. Since S1P does not activate the PI3K/Akt pathway in these cells, inhibition of this pathway leading to a pro-survival effect is probably linked to its basal activation independent of S1P. Microarray analysis showed that S1P modifies the expression of a large number of genes (>1,000) in hESCs with an up-regulation of anti-apoptotic, cell cycle progression and cell adhesion genes, and

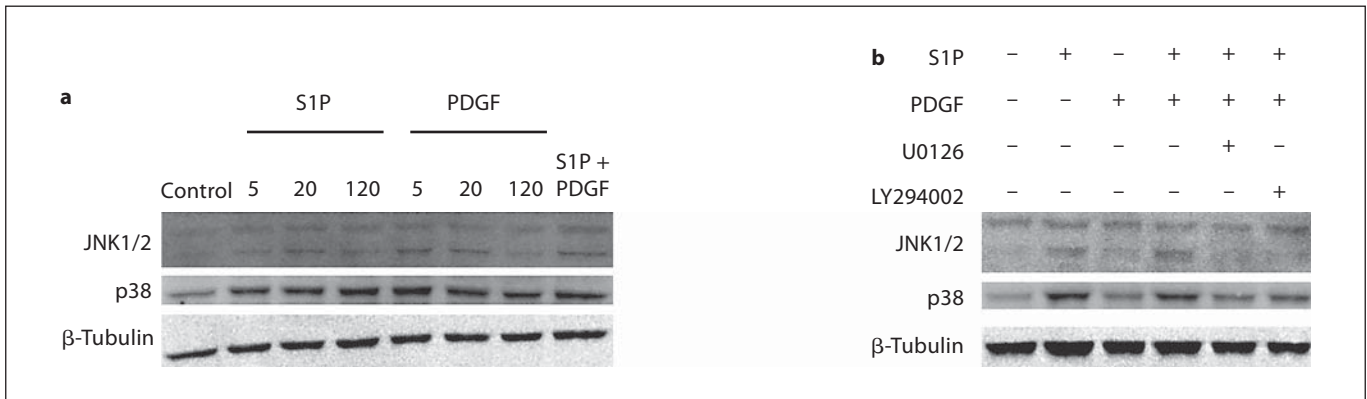


Fig. 2. S1P and PDGF induce the phosphorylation of JNK1/2 and p38 in hESC ($n > 3$), in an ERK1/2-dependent manner. **a** S1P (10 μ M) and PDGF (20 ng/ml) stimulate JNK1/2 and p38 phosphorylation in hESC. 5, 20, 120 = Time of activation in minutes. **b** Co-incubation of S1P + PDGF (20 min) induces phosphorylation of

JNK1/2 and p38 in hESC. JNK1/2 and p38 phosphorylation are regulated by ERK1/2 as U0126 reduces JNK and p38 phosphorylation induced by S1P/PDGF. The membranes were stripped and re-blotted with β -tubulin as a loading control.

down-regulation of pro-apoptotic genes, suggesting that S1P is an important factor in hESC maintenance [57]. However, S1P also down-regulates pluripotency genes in particular *nanog* and *Oct-4* [57], which suggests that S1P by itself is not sufficient to maintain hESCs undifferentiated. In an attempt to identify compounds within hESC media that benefit hESC maintenance, Garcia-Gonzalo and Belmonte [59] identified that albumin-associated lipids present in media stimulate hESC renewal, yet did not observe the obvious effect of either LPA or S1P on hESCs, which confirms our findings that sole application of S1P or LPA does not maintain hESCs in culture [53].

From the studies discussed above it is clear that LPA and S1P have different effects on mouse and human ESCs. This is not surprising as the signalling pathways involved in maintenance or differentiation in both cells are different. Indeed, in both cell types S1P stimulates the ERK1/2 pathway, a signalling pathway generally involved in the differentiation of mESCs and in the maintenance of hESCs. Whether this reflects a variation between species or a difference in the cells used to generate ESC lines needs to be further determined.

Ceramide, while not a lysophospholipid, is a precursor to S1P and has been observed to have effects on hESCs, although the true role of this lipid in hESC regulation remains somewhat controversial. Indeed, as is observed in the mouse, undifferentiated hESCs within EBs are sensitive to ceramide and undergo apoptosis following incubation with ceramide [51]. Another recent study showed, however, that colonies of undifferentiated hESCs are re-

sistant to ceramide-induced apoptosis while more differentiated hESCs within the colonies are not [60]. Indeed, the authors of this latter study exploited this characteristic to successfully maintain hESCs in culture by adding ceramide [60]. Thus, in 2 different assays using hESCs, the sensitivity towards ceramide appeared to be different. These contradictory results might be due to the different culture systems used in these studies, and it is tempting to speculate that it may reflect a variation in ceramide metabolism, including cellular SphK activity, depending on the environment in which hESCs are cultivated.

Induced Pluripotent Stem Cells

In 2006, a novel technology was developed whereby 4–6 genes are introduced into an adult somatic cell resulting in its reprogramming into an ‘embryonic-like’ or pluripotent cell, named induced pluripotent stem cell (iPS) [61–66]. Derivation of human iPS from diseased cells allows the generation of hESC-like cells, without having to derive hESCs from fertilised oocytes or cloning. As iPS can be generated from biopsies, these cells open new avenues to study diseases, and overcome rejection if injected into the source patients. In theory, ESCs and iPSs are able to differentiate into all cell types of the body. For this reason, they generate great hope for human therapy/regenerative medicine, as well as being useful as a human cell model for drug discovery. Our data indicate that iPSs express the mRNA for S1P_{1,3} and LPA_{1–4}, which differs from hESC expression (fig. 3). This might suggest different roles of S1P and LPA in these cells than in hESCs

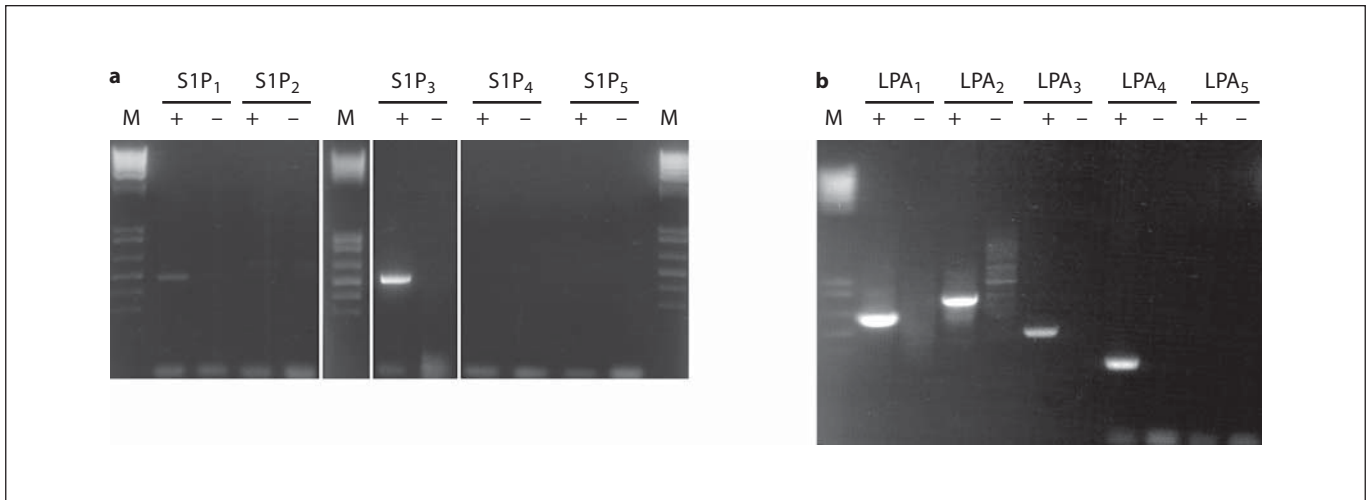


Fig. 3. S1P and LPA receptor mRNA are expressed in human iPS. RT-PCR for S1P (**a**) and LPA (**b**) receptors with (+) or without (-) RT in human iPS. M = DNA ladder. Expected sizes: S1P₁ 480 bp; S1P₂ 502 bp; S1P₃ 505 bp; S1P₄ 701 bp; S1P₅ 460 bp; LPA₁ 621 bp;

LPA₂ 775 bp; LPA₃ 482 bp; LPA₄ 318 bp, and LPA₅ 185 bp. RT-PCR were performed as described by Pebay et al. [53] and Dottori et al. [55]. Data are representative results of independent experiments performed on 2 different iPS cell lines: hiPS1 and ESCL4.

or be a consequence of a non-total reprogramming of fibroblasts into iPSs. However, their biological effects in iPSs have not yet been investigated. These data also indicate phenotypic variation between iPSs and hESCs.

LPA/S1P Signalling in Neural Stem/Progenitor and Oligodendrocyte Progenitor Cells

LPA/S1P Receptor Expression in Neural Stem/Progenitor and Oligodendrocyte Progenitor Cells

S1P and LPA receptors are expressed in neuroblasts and NS/PCs and both ligands display a range of effects on these cells. It seems, however, that receptor expression varies between species, developmental stage and origin of the NS/PCs. Rat embryonic hippocampal NS/PCs express S1P_{1-3,5} and undetectable levels of S1P₄ [67] and immortalised rat embryonic hippocampal progenitor H19-7 cells express LPA_{1,4} [68]. Mouse and rat embryonic forebrain NS/PCs express all 5 S1P receptors (S1P₁₋₅), although quantitative PCR showed some variation in the expression of these receptors [69, 70]. Indeed, mouse embryonic forebrain NS/PCs predominantly express S1P_{1,2}, lower levels of S1P₃ and weak levels of S1P_{4,5} [70], while rat embryonic forebrain NS/PCs predominantly express S1P₁, lower levels of S1P_{2,4} and weak levels of S1P_{3,5} [69]. Rat embryonic forebrain NS/PCs also express LPA_{2>1,3},

but no data are available on the expression of other LPA receptors [69]. Seven-day postnatal mouse forebrain NS/PCs express LPA₁₋₃ [71]. In the human, hESC-derived NS/PCs express LPA₁₋₅ and S1P_{1,3} (fig. 4a), although their relative abundance is not yet known [55], and hESC-derived neuroepithelial cell line (NEP) – a stable line that resembles hESC-derived NS/PCs and grows under adherent conditions [72] – expresses LPA_{1,2,4>5}, S1P_{1-3>5} and undetectable levels of LPA₃ and S1P₄ [73].

From these studies it is clear that differential receptor expression can partially explain the variation in cellular effect of LPA or S1P on NS/PCs, as these different receptors signal through different G proteins. In particular, LPA_{4,5} are hypothesised to stimulate G_s as they stimulate adenylate cyclase signalling in some cell types [38, 74, 75], thus their absence would potentially remove the possibility of G_s/cAMP production. The absence of other receptor subtypes might have fewer consequences on cell signalling. For instance, S1P₄ or LPA₃ show redundancy of signalling with other receptors; thus, their absence (as observed in human NEP) is likely not to be associated with a lack of signalling.

Oligodendrocyte Progenitor Cells

As observed in NS/PCs, data obtained in OPCs suggest variation in receptor expression between species. LPA₁ and S1P_{5>1-3} are expressed by embryonic and adult rodent OPCs [76–79], while human fetal OPCs express

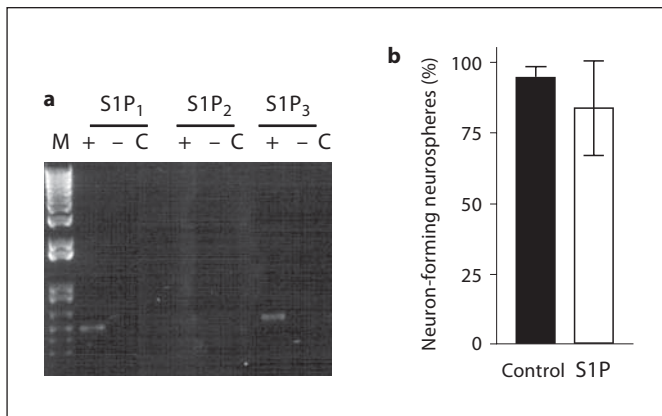


Fig. 4. S1P receptor mRNA expressed in hESC-derived NS/PC and S1P does not inhibit their neuronal differentiation. **a** RT-PCR for S1P receptors with (+) or without (-) RT in hESC-derived neurospheres. M = DNA ladder; C = control PCR. Expected sizes as in the legend to figure 3. Data are representative results of at least 3 independent experiments. **b** Quantification of neuron-forming neurospheres from hESC-derived neurospheres plated onto laminin and incubated in the absence (control) or in the presence of S1P (10 μ M) for 5 days. Quantification was done by counting the number of spheres from which neuronal outgrowth was observable. In some cases, neurospheres failed to attach, independent of the treatments, and these floating neurospheres were not taken into consideration for quantification. Data are expressed as the means \pm SEM from at least 3 independent experiments. $p > 0.05$ ($n = 3$) by t test.

high levels of S1P₁, low levels of S1P_{5>3} and undetectable levels of S1P₄ [80]. LPA receptor expression in human OPCs has not yet been described. In a mESC-derived cell line hypothesised to be OPCs, due to the expression of the marker A2B5 (which is however also found on other glial cells), S1P₁ was also shown to be expressed [50].

LPA/S1P Signalling in the Developing CNS

In the developing central and peripheral nervous systems, LPA and S1P have been shown to target endothelial cells, microglia [81, 82], astrocytes [83–87], oligodendrocytes [88], Schwann cells [89], neurons [90] and stem cells [30, 53, 55, 91]. Studies suggest that during development, LPA stimulates neuronal differentiation of embryonic cortical neuroblasts, neural progenitors and early cortical neurons [68, 71, 92, 93]. LPA can be produced and released by post-mitotic neurons, oligodendrocytes [77, 94] and Schwann cells [91, 95], and it is now hypothesised that LPA is an important factor for cortical neurogenesis by ‘guiding’ the migration and differentiation of neuroblasts, then neurons, from the ventricular zone to their final destina-

tion during development [92, 93, 95, 96]. Data also suggest a local synthesis and release of S1P within the CNS. For example, cerebellar astrocytes have been shown to release S1P in response to basic fibroblast growth factor (bFGF), NS/PCs express SphK1 and SphK2, and glutamate induces the release of high levels of S1P by OPCs [67, 78, 97]. Little is known of the effect of S1P in neuroblasts and NS/PCs, and reports on the effect of LPA often appear contradictory. Indeed, effects vary between NS/PCs of embryonic or adult origin, and between species, which might either reflect fundamental differences in LPA signalling between species or may simply be due to the examination of different cell populations and/or variation in the LPA concentration used. Yet, the effects of S1P and LPA on neural progenitor cells have been described on differentiation, proliferation, morphological changes and migration.

Neuroblasts

LPA stimulates neuronal differentiation of mouse cerebral cortex neuroblasts, NS/PCs and early neurons, possibly via LPA₁/G_i proteins [92, 93, 95]. Proliferation of mouse cerebral cortex neuroblasts in response to LPA was observed in vitro but not retrieved in vivo [92]. Morphological rearrangements are also induced by LPA. These include cell rounding, membrane retraction, formation of retraction fibres and cluster compaction [92, 95, 98, 99], through LPA₁-mediated Rho activation [95]. Notably, no comparable effects were observed in these cells with S1P [95]. Interestingly, LPA also depolarises mouse embryonic cortical neuroblasts by increasing ionic conductances [100], which in turn activates the electrical responses preceding GABA and L-glutamate signalling [100].

Neural Stem/Progenitor Cells

NS/PCs can be maintained in culture as neurospheres in the presence of bFGF and EGF [101, 102]. Further differentiation of NS/PCs into mature neurons and glial cell types can be achieved by growth of the NS/PCs on laminin or fibronectin substrates, respectively, and culturing without growth factors. LPA inhibits mouse embryonic cortical NS/PC growth as neurospheres, at least partially via transiently increasing cell death [93]. Yet, in neurospheres from 7-day postnatal mice, LPA induces clonal generation of neurospheres through activation of LPA₁, supports neurosphere growth and proliferation through LPA_{1,3} [71], and in rat cortical NS/PCs, LPA stimulates (<1 μ M) or inhibits (at concentrations of >1 μ M) proliferation, while also promoting neuronal differentiation and migration [103]. However, in human NS/PCs derived

from hESCs, pathological concentrations of LPA (10 μM) inhibits neurosphere formation without modifying proliferation or apoptosis [55], while LPA and S1P (<0.1 μM) stimulate cell growth of NEP probably through G_i transactivation of EGF and activation of the ERK1/2 pathway [73]. In rat hippocampal NS/PCs, LPA does not affect proliferation, probably due to the fact that it does not activate ERK1/2 in these cells. LPA does, however, induce morphological rearrangements in these cells, an effect dependent on Rho-associated kinases [67], while S1P stimulates their proliferation through G_i -dependent ERK1/2 activation, increases telomerase activity, induces Rho-mediated morphological changes, and increases expression of bFGF and *Egr-1* (early growth response-1) [67]. Furthermore, LPA promotes neuronal differentiation of mouse embryonic cortical NS/PCs [93] and of the immortalised embryonic hippocampal progenitor cell line H19-7, possibly through G_s and cAMP signalling [68]. LPA, however, inhibits hESC-derived NS/PC neuronal differentiation, while maintaining glial differentiation [55], a reversible, receptor-mediated effect (at least by LPA_{1/3}) that is dependent on PI3K/Akt and Rho/ROCK [55]. In NEP, in addition to stimulating cell growth, LPA and S1P also induce reversible cell rounding through Rho/ROCK [73], while no obvious effect of S1P on hESC-derived NS/PC differentiation was observed (fig. 4b). Thus, although similar, hESC-derived NS/PCs and NEP do not show similar responses to LPA and S1P. Further work is required to examine if these apparent differences are real, or are simply reflective of the different conditions under which these studies have been performed.

LPA/S1P Signalling in Oligodendrocyte Progenitors

One essential aspect of oligodendrocyte maturation is the formation of processes involved in the migration of OPCs and the myelination of neurons [104]. LPA and S1P both target OPCs and inhibit this aspect of oligodendrocyte maturation. Through the activation of the Rho pathway by LPA₁ and S1P₅, respectively, LPA and S1P induce process retraction [76, 77]. LPA and S1P appear to activate different signalling pathways in OPCs as (i) only S1P induces membrane ruffling [76], and (ii) S1P inhibits the integrin-driven migration of OPCs through S1P₅/Rho [78]. S1P₅ is preferentially expressed in the oligodendrocytic lineage [79] and is responsible for the S1P inhibition of rodent OPC migration [78]. In human OPCs, the S1P analogue FTY720 regulates S1P receptor expression, leading to temporally regulated biological effects: FTY720 first induces process retraction and inhibition of differentiation through the S1P_{5,3}/Rho

pathway, and secondly stimulates process extension, cell survival in a S1P₁/ERK1/2-dependent manner [80, 105, 106].

Studies also indicate cross-talk between neurotrophin and S1P signalling in OPCs. Neurotrophin-3 (NT-3) stimulates the proliferation and survival of OPCs via the phosphorylation of cAMP-response element-binding protein (CREB) [107], a signalling pathway that is partially controlled by SphK [94]. S1P also stimulates CREB phosphorylation, an effect mediated by the protein kinase C/ERK pathway [94]. As dihydro-S1P mimics the effect of S1P in OPCs, it was proposed that S1P acts through the binding of its specific receptors after its release from cells following NT-3 activation of SphK1 [94]. Lastly, Bieberich and colleagues identified a small subpopulation of NS/PCs, named NPC2 cells, that have high levels of PAR-4. Notably, S1P rescues these cells (which may be OPCs) from ceramide-induced apoptosis, probably through S1P₁ [50] providing further evidence that the balance between ceramide and S1P may be an important influence on stem cell biology and differentiation protocols [50, 51, 108–110].

LPA/S1P Signalling in the Injured CNS through Neural Stem/Progenitor Cells

To date, little has been examined regarding the effects of LPA and S1P in the adult CNS, despite data strongly suggesting that neural responses to LPA and S1P stimuli are likely to significantly influence the amount of ensuing damage or repair. Following events which damage the blood-brain barrier, 'LPA-like activity' is increased within the cerebrospinal fluid and levels of LPA within the CNS are hypothesised to increase up to 10 μM [91, 111–113]. Normally undetectable, levels of the LPA-producing enzyme autotaxin increase in astrocytes neighbouring a lesion of the adult brain [114], supporting a role for LPA in brain injury responses. LPA injections into mouse brain induce astrocyte reactivity at the site of the injury [115], while in the spinal cord following trauma, LPA induces neuropathic pain and demyelination [116–121]. LPA can stimulate astrocytic proliferation [87, 111] and, depending on its concentration, it can promote death of hippocampal neurons by apoptosis (1 μM) or by necrosis (10 μM) [122]. Moreover, LPA mediates microglial activation [81] and is cytotoxic to the neuromicrovascular endothelium [123]. Furthermore, data suggest an important role of LPA and S1P on embryonic and adult NS/PC differentiation following trauma. For example, our studies with embryonic derived-NS/PCs show that a pathological concentration of LPA (10 μM) inhibits neuronal dif-

differentiation of human NS/PCs, while lower concentrations do not [55]. This suggests that the presence of high levels of LPA within the CNS following an injury inhibits endogenous neuronal regeneration and maintains gliogenesis. S1P levels also increase in the CNS following spinal cord injury, brain injury or disruption of the blood-brain barrier [69, 70, 113, 124]. Following brain or spinal cord injury, S1P signalling is reported to mediate migration of NS/PCs towards the lesion sites [69, 70]. Indeed, S1P is a potent chemoattractant of NS/PCs and induces the migration of endogenous NS/PCs towards brain-injury sites [70], and of transplanted NS/PCs toward spinal cord injury sites via G_i and the Rho pathway [69]. Exogenous NS/PC migration is also induced by LPA but to a lesser extent [69].

LPA and S1P are also modulators of various cancers and can influence cancer stem cell biology. In humans, the CD133-positive cancer stem cells which represent a subpopulation of cells within glioblastoma [125] are highly tumorigenic, show resistance to chemotherapy [126] and are responsive to LPA and S1P. These cells express all S1P receptors and at least LPA₁₋₃, with high levels of LPA_{1,3} and S1P_{1,2,4} *in vitro* and high levels of LPA₁ and S1P_{1,2} *in vivo*, a variation in receptor expression that might be due to a paracrine regulation within the tumour [125]. Notably, both SphK1 and autotaxin are elevated in glioblastomas [127–131], and their products, S1P and LPA, have been shown to stimulate migration of these cells [131–133], which is likely to be responsible, at least in part, for the tumour invasiveness of the cancer stem cells [125].

Conclusions

While still an emerging area of research, there is now considerable evidence pointing to important roles of lysophospholipids in the regulation of stem cell biology. As described above, LPA and S1P have been shown to modulate proliferation, survival, differentiation and migration of embryonic and neural stem cells. This diverse array of biological effects clearly illustrates the complexity of lysophospholipid signalling. Indeed, these lysophospholipids can act both intracellularly and extracellularly, can bind to multiple receptors coupled to various G proteins to directly activate or inhibit various signalling pathways, and also cross-talk with other cytokine and growth factor receptors. It is apparent that stem and progenitor cells express different lysophospholipid receptors not only depending on their source of origin, but also on the conditions used for their isolation and/or culture. This illustrates the difficult task ahead of transposing to the human system the data obtained in both developing or adult animals and cultured stem and progenitor cells.

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