Stem Cell Biology and Regenerative Medicine

Alice Pébay
Raymond C.B. Wong *Editors*

Lipidomics of Stem Cells

Stem Cell Biology and Regenerative Medicine

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Lipidomics of Stem Cells

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Preface

This volume of *Stem Cell Biology and Regenerative Medicine* aims at covering the current knowledge on the role of lipids in stem cell pluripotency and differentiation. We would like to thank all the authors to this volume who have shared their expertise.

We also wish to thank Dr. Kursad Turksen for his support during the process of compiling this book. Finally, a special thank you goes to Michael Koy for his help during the preparation of the volume.

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Chapter 1
Lysophosphatidic Acid and Sphingosine-1-Phosphate in Pluripotent Stem Cells

Grace E. Lidgerwood and Alice Pébay

Abbreviations

ABC	ATP-binding cassette
ATX	Autotaxin
ENNP2	Ectonucleotide pyrophosphatase phosphodiesterase 2
ERK	Extracellular signal-regulated kinase
HDAC	Histone deacetylase
hESC	Human embryonic stem cell
iPSC	Induced pluripotent stem cell
JNK	c-jun N-terminal kinase
LPA	Lysphosphatidic acid
MAP	Mitogen-activated protein
mESC	Mouse embryonic stem cell
PDGF	Platelet-derived growth factor
PI3K	Phosphoinositide 3-kinase
PPAR	Peroxisome proliferator-activated receptor
S1P	Sphingosine-1-phosphate
SPhK	Sphingosine kinase
TRAF2	TNF receptor-associated factor 2
VEGF	Vascular endothelial growth factor

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1.1 Introduction

Lipidomics refers to the analysis of lipids in cells, tissues, or organisms. Lipids are one of the main classes of biomolecules necessary to life, yet are probably the least understood and studied biomolecules. It is estimated that there are between 9,000 and 100,000 different lipid species [1, 2]. This massive range reflects how little is known about this class of biomolecules. Few techniques are currently available to the study of lipids, and it is very difficult to isolate and analyze lipids, explaining why lipidomics somehow lags behind the study of other biomolecules. Lipids are the major compounds of the biological membranes that serve as the physical barrier, giving structural integrity to the cell and its components. They also play an important metabolic function in terms of energy storage. Lipids are also integral to membrane trafficking and can be found in vesicles such as exosomes. Lipids with cell signaling functions are often referred to as bioactive lipids, as opposed to lipids that form the structural composition of cell membranes or those used for energy, and have an array of biological functions, including mediating inflammation; regulating cell growth and polarity; and determining cell fate decisions. This essential signaling feature of bioactive lipids occurs in a variety of different pathways; lipids can engage with specific receptors to activate a cascade of downstream signaling pathways, or through indirect means, i.e., via membrane trafficking or as lipid rafts.

1.2 Lipid Homeostasis in Stem Cell Biology

A stem cell is a cell that is capable of self-renewing by undergoing indefinite symmetrical cell divisions, giving rise to daughter cells that are genetically identical to the original parent cell. Under the right conditions, stem cells can also differentiate into specialized cells that have specific functions in the body. Adult stem cells are generally of multipotent potential, meaning they are capable of differentiating into restricted lineages. Pluripotent stem cells, on the other hand, are capable of giving rise to all cell types of the body. There are two main sources of pluripotent stem cells: embryonic stem cells (ESCs), which are derived from the inner cell mass of a preimplantation blastocyst; and induced pluripotent stem cells (iPSCs), somatic cells that have been reprogrammed into a pluripotent state, and exhibit functional similarities to ESCs. Pluripotency is maintained by the expression of particular genes, which is intricately controlled by the homeostasis of a range of regulatory signaling molecules and epigenetic factors. Subtle changes in cellular conditions ultimately determine the fate of pluripotent stem cells. Historically, scientists have focused on the role of signaling proteins and genetic factors in the maintenance of pluripotency; however, more recently, signaling lipids have surfaced as potential regulators of stem cell maintenance and differentiation.

Lipid homeostasis is fundamental to development and cellular homeostasis, and lipid dysregulations can lead to developmental abnormalities as well as

neurodegeneration [3–5]. It is likely that changes in the lipidomic signature of a cell from pluripotency to differentiation will reflect a change in substrate availability during these events and may also give rise to a predictive model of differentiation and maturity. For instance, there is evidence that specific lipids play fundamental roles in neural development [6–8] but less is known about the general profile of lipids in pluripotency and upon differentiation. There are in fact a limited number of large lipidomic studies that have been performed within the stem cell field. Nonetheless, there is some suggestion that depending on their level of pluripotency or differentiation, cells will show a different distribution of heterogenous lipids [9]. Further, the lipidome is also modified upon mouse ESC differentiation [10]. Interestingly, Wang et al. [11] demonstrated in a landmark publication that in *C. elegans*, germ line stem cell longevity is regulated by an active control of lipid metabolism [11]. Further, the lipidomic profiling of mouse retinal stem cells identified a distinct glycerophospholipid signature, which when altered, participates in the regulation of proliferation or differentiation [12]. Similarly, the peroxisome proliferator-activated receptor (PPAR) pathway acts as a metabolic switch to control hematopoietic stem cell maintenance or differentiation, by regulating the oxidation of fatty acids [13], thus suggesting a direct impact of lipids on cell fate. Human iPSCs are composed of less saturated fatty acids than human (h)ESCs, which may indicate metabolic differences in these two cell types [14]. This exemplifies how lipid homeostasis is most likely fundamental to pluripotency and differentiation.

1.3 LPA and S1P Synthesis and Degradation

Lysophospholipids are bioactive lipids consisting of one *O*-acyl chain, generated by the hydrolytic cleavage of fatty acids from glycerophospholipids by phospholipases. Two main categories of lipids form lysophospholipids: those derived from glycerol, glycerophospholipids (including LPA) and those with a sphingomyelin backbone, sphingolipids (including S1P). Both these classes of lipids play an integral role in cell fate, including in regulating pluripotency and differentiation of various types of stem cells. LPA and S1P are the most characterized lysophospholipids in terms of effects in pluripotent stem cells, and will thus be the major focus of this chapter.

1.3.1 LPA

LPA can be synthesized and degraded through a variety of pathways [8, 15]. Autotaxin/ectonucleotide pyrophosphatase phosphodiesterase 2 (ENNP2) is responsible for most of the production of extracellular LPA. This secreted enzyme has a

lysophospholipase D domain able to cleave lysophospholipids, in particular lysophosphatidylcholine, into LPA. Other enzymes can also generate extracellular LPA: secreted phospholipases A1 and A2, which can deacylate phosphatidic acid. Intracellular LPA, on the other hand, can be generated by other enzymatic pathways that include activities of intracellular phospholipases A1 and A2; glycerol 3-phosphate acyltransferase, which acylates glycerol 3-phosphate; or monoacylglycerol kinase, which phosphorylates monoacylglycerol. LPA degradation is then mediated by lipid phosphate phosphatases 1–3, which dephosphorylates LPA to monoacylglycerol.

1.3.2 S1P

Sphingolipids are acyl (fatty acid) derivatives of the amino alcohol, sphingosine, and encompass a range of bioactive lipids, including S1P. In contrast to LPA synthesis, S1P can only be generated by one pathway, involving the phosphorylation of sphingosine by sphingosine kinases (SphK) 1 and 2. S1P can then be degraded by S1P lyase, or dephosphorylated into sphingosine by S1P phosphatases and nonspecific lipid phosphatases, or converted to ceramide by ceramide synthase [15, 16]. S1P is synthesized intracellularly and thus needs to be excreted in order to act as an extracellular ligand. This export is likely to occur through ATP-binding cassette (ABC) transporters [16]. S1P is also present in the nucleus and in the mitochondria, where it is synthesized by SphK2 [17, 18].

1.4 LPA and S1P Signaling

LPA and S1P act extracellularly mainly through the binding to their specific G protein-coupled receptors. There are currently six confirmed LPA receptors (LPA_{1-6}) and five S1P receptors ($S1P_{1-5}$) [19]. Other extracellular receptors have been implicated as LPA receptors, including the purinergic receptors P_2Y_5 and P_2Y_{10}, GPR87 and the TRPV1 channel [8]. LPA and S1P receptors are known to act through G_q and $G_{12/13}$, G_i and potentially G_s, to modulate multiple signaling pathways including: stimulation of phospholipase C/protein kinase C and modification in intracellular calcium concentration; stimulation of the phosphoinositide 3-kinase (PI3K)/AKT pathway; stimulation of Ras/mitogen-activated protein (MAP) kinase pathways including of extracellular signal-regulated kinases (ERK) 1/2; inhibition and potential stimulation of adenylate cyclase pathways; activation of small G proteins and subsequent stimulation of the Rho/ROCK pathway; and activation of phospholipases A_2 and D [19].

Both LPA and S1P can thus act as extracellular mediators by binding their cellular membrane receptors, but they can also act as intracellular receptors. Some research indeed suggests that the nuclear receptor PPARγ can also bind LPA [8]. As for S1P, it is now clearly demonstrated that it is an intracellular nuclear mediator, with direct interaction with key molecules that are not S1P receptors [20].

Intracellularly, S1P is known to counteract the pro-apoptotic effects of ceramide, contributing to the S1P-ceramide rheostat [20]. Intracellular S1P has also been shown to modulate NF-κB signaling by interacting with protein kinase Cδ and TNF receptor-associated factor 2 (TRAF2) [20]. It can also directly interact with events controlling mitochondrial respiration [18]. Finally, within the nucleus, S1P has been shown to bind and inhibit histone deacetylases (HDACs) 1/2, which most likely has consequences on gene regulation and epigenetics [17]. This could be particularly relevant to pluripotency.

Given the complexity of LPA and S1P signaling, it is not surprising that these molecules induce pleiotropic biological effects in different cells, including stem cells [21, 22].

1.5 Role of LPA and S1P in Pluripotent Stem Cells

LPA and S1P have been implicated in events regulating survival, autophagy, apoptosis, proliferation, differentiation, cytoskeleton rearrangements, polarity, and migration. Lysophospholipids also control events of pluripotency and differentiation in both adult and embryonic stem cells and in various species (as reviewed in [8, 15, 23, 24]). Both mouse and human pluripotent stem cells express LPA and S1P receptors, with some variations. Mouse ESCs express $LPA_{1,2,3}$ [25] and $S1P_{1–5}$ [26–29] although $S1P_4$ expression depends on the mESC lines [30]. Human ESCs and iPSCs express $LPA_{1–5}$ and $S1P_{1–5}$ [23, 31–33] with some expression variation depending in cell lines, as observed with mouse ESCs. These differences could be artifacts of cell culture methods. Although unlikely - given the redundancy in signaling pathways modulated by the various receptors - the difference in receptor expression between pluripotent stem cell lines might indicate some potential variation in these bioactive lipids' cellular effects.

Both LPA [25] and S1P [29, 30] stimulate proliferation of mESCs. LPA's effect is dependent on the activation of the phospholipase C pathway, leading to modifications of intracellular calcium concentration, itself inducing expression of the early gene *c-myc* and subsequent proliferation [25]. LPA also induces Erk phosphorylation and downstream c-fos activation in the pluripotent stem cells [34]. Given the role of c-myc in pluripotency and reprogramming of somatic cells into iPSCs [35], it is interesting to note that LPA is able to induce its expression in ESCs. Likewise, S1P stimulates mESC proliferation, at least through its receptor-mediated activation of the Erk pathway [29, 30]. Other pathways might intervene. In particular, Ryu et al. [29] suggest that S1P promotes mESC proliferation by the $S1P_{1/3}$-induced transactivation of the vascular endothelial growth factor (VEGF) receptor, Flk-1, and subsequent phosphorylation of Jnk and Erk [29]. Together with the demonstration that S1P induces VEGF expression in mESCs [29], this data suggests an important interaction between S1P and VEGF in mESC pluripotency. Finally, the knocking down of S1P lyase in mESCs is accompanied by a large increase in S1P levels, increased proliferation and expression of the mouse pluripotency markers sse4 and

oct4, as well as an increase in stat3 signaling, all suggestive that endogenous S1P metabolism is highly regulated in mESCs and is key to pluripotency [28].

In hESCs, we reported that we did not observed an effect of LPA alone (up to 10 μM) on their maintenance [31], which was similarly reported by others using a different culture medium [36]. LPA has however been described as blocking Wnt pro-differentiation effects in hESCs [36]. Of note, it was also described that low concentrations of LPA (up to 100 nM) slightly increases the number of pluripotent cells in conditions favoring differentiation (mTeSR without basic fibroblast growth factor), while 1 μM induces death of hESCs [32]. This data is at odds with the previous reports, which could be partially explained by the fact that LPA was reconstituted and prepared in water in place of solvents (generally chloroform or ethanol/water) necessary for LPA solubilization. Together, these data suggest that LPA may be important for the maintenance of pluripotency, most likely as a "counter actor," an anti-differentiation agent, rather than a direct pro-pluripotency factor.

Recently, LPA was shown to modulate the Hippo pathway in both hESCs and human iPSCs, by activating YAP/TAZ [37, 38]. This is interesting in terms of pluripotency and differentiation, as the Hippo pathway is fundamental to development and is key to stem cell pluripotency and differentiation (for review of the pathway, see [39]). Indeed, when active, the YAP/TAZ transcriptions factors would be involved in self-renewal of hESCs and iPSCs, while inactivation of the pathway was shown to be linked to differentiation [37]. Interestingly, the activation of YAP by LPA results in the stimulation of a naïve state in hESCs and human iPSCs [38], allowing the generation of transgene-free human naïve pluripotent stem cells, clearly indicative of a fundamental role of LPA in human pluripotency.

On the other hand, S1P, in combination with platelet-derived growth factor (PDGF), was shown to maintain hESCs undifferentiated, in G_i-, ERK-, and SphK-dependent mechanisms [31]. This maintenance of pluripotency was observed with cells cultivated on feeder and feeder-free, and in the absence of serum, thus demonstrating a direct effect of S1P on hESCs. Interestingly, S1P alone was not able to maintain hESCs undifferentiated, and PDGF was shown to stimulate SphK, thus allowing the generation of intracellular S1P [31]. It is thus feasible that the presence of both extracellular S1P- and intracellular S1P-mediated effects contribute to the maintenance of pluripotency and further work to clarify this point would be interesting. S1P was also shown to be anti-apoptotic in hESCs, through the phosphorylation of ERK 1/2, but independent of the PI3K pathway [40]. S1P can also induce the phosphorylation of p38 and to a lesser extent of c-jun N-terminal kinases (JNK) in hESCs, but the significance of these activated pathways remains to be established [23]. Finally, S1P does not induce intracellular calcium modification, suggesting that the phospholipase C pathway is not essential to hESC pluripotency and survival [40]. This pro-survival effect of S1P was also observed by an increased expression of anti-apoptotic genes and cell cycle-related genes, and a down-regulation of pro-apoptotic genes [41].

Little is known on the basal levels of LPA and S1P in pluripotent stem cells. High performance liquid chromatography—mass spectrometry revealed that many sphingolipid intermediates are present in hESCs, in particular ceramide and low levels of

intracellular S1P [42]. It was recently demonstrated that hESCs and human iPSCs express cilia that are regulated by the ceramide/sphingomyelinase pathway [43]. Given the close relationship between ceramide and S1P, it is possible that intracellular S1P might also be involved in ciliogenesis, a fundamental process of developing cells.

1.6 Discussion and Conclusion

Little is known of the role of lipids, their interactions, catabolism, metabolism and how these modulate many diverse biological processes, including in stem cells. The world of lipids is complex, in terms of functions, diversity, and numbers, and is probably the least understood "-ome" of biology. With today's technology and given the extremely large numbers of lipids per cell, it is still not possible to assess the entire lipidome of a cell. However, lipidomics is now emerging because tools and strategies used for genomics and proteomics are being applied to the study of lipids. For instance, high performance liquid chromatography, electrospray ionization mass spectrometry, coupled with bioinformatic analysis will allow for large-scale system-level analysis of lipids and pathways involved [44]. These techniques might help answer important questions, such as: are there modifications in the lipidome of cells upon cellular fate? If so, are these a consequence of the cellular transition or are they a driving force behind change?

In terms of signaling lipids, it is clear that these play fundamental role in stem cell biology. In particular, LPA and S1P modulate various effects in various stem cells, both pluripotent and multipotent (as reviewed in [15]). In pluripotent stem cells, there seems to be some difference in effects of LPA and S1P between species, but it is clear that these molecules positively influence pluripotency and survival. A further understanding of the role played by intracellular S1P in pluripotency, epigenetics, and on the Hippo pathway would most likely be very informative. Likewise, a clearer picture of the interactions between LPA and Wnt signaling in pluripotent stem cells and upon differentiation would provide new knowledge in our understanding of the complexity of lysolipid signaling in pluripotency.

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Chapter 2
Morphogenetic Sphingolipids in Stem Cell Differentiation and Embryo Development

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Abbreviations

Akt	AK strain transforming (Akt kinase)
aPKC	Atypical PKC
C1P	Ceramide-1-phosphate
CECs	Ceramide-enriched compartments
EGF	Endothelial growth factor
ERK	Extracellular regulated kinase
ES cell	Embryonic stem cell
EV	Extracellular vesicle
FB1	Fumonisin B1
FGF-2	Fibroblast growth factor 2
FTY720	Fingolimod
GPCR	G protein-coupled receptor
Grp94	Glucose-regulated protein 94
GSK3	Glycogen synthase kinase 3
GSLs	Glycosphingolipids
HDAC	Histone deacetylase
hESC	Human ES cell
HSP90	Heat shock protein 90
iPSC	Induced pluripotent stem cell
Jak	Janus kinase
LIF	Leukemia inhibitory factor
MAPK	Mitogen-activated protein kinase
mESC	Mouse (murine) ES cell

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NPC	Neural precursor cell
nSMase	Neutral sphingomyelinase
OPC	Oligodendrocyte precursor cells
PAR-4	Prostate apoptosis response 4
PDGF	Platelet-derived growth factor
PDMP	*N*-[2-hydroxy-1-(4-morpholinylmethyl)-2-phenylethyl]-decanamide
PHB2	Prohibitin 2
PI3K	Phosphatidyl inositol 3 kinase
PIP	Phosphatidyl inositol phosphate
PKC	Protein kinase C
PLC	Phospholipase C
PP2a	Protein phosphatase 2a
S18	*N*-oleoyl serinol
S1P	Sphingosine-1-phosphate
Shh	Sonic hedgehog
SphK	Sphingosine kinase
SPL	S1P lyase
Spns2	Spinster homolog 2
Stat3	Signal transducer and activator of transcription 3
Wnt	Wingless type MMTV

2.1 Ceramide and Its Derivatives

In this section, we will focus on the function of ceramide and derivatives known to regulate stem cell differentiation, namely, sphingosine-1-phosphate (S1P), ceramide-1-phosphate (C1P), and glycosphingolipids (GSLs) (Fig. 2.1). We will not discuss sphingolipid metabolism or the function of sphingolipids in general cell-signaling pathways. There are excellent reviews and the reader is encouraged to attend to these resources [1, 2]. Instead, we will highlight most recent studies showing the function of sphingolipids in cell-signaling pathways critical for regulation of cell polarity and morphogenesis as part of the stem cell differentiation program.

2.1.1 Ceramide and Ceramide-Enriched Compartments

A morphogenetic lipid will induce a specific stem cell differentiation program and regulate embryo development and morphogenesis. We have proposed that ceramide is such a morphogenetic lipid based on the observation that it is critical for the apicobasal patterning of the primitive ectoderm in embryonic stem (ES) cell-derived embryoid bodies and for promoting neural differentiation [2–6]. Compartmentalization into ceramide-enriched compartments, CECs, allows for localized metabolic release